

B<sup>1</sup>  
--11. A mutant barnase gene comprising one of the nucleotide sequences which encode the amino acid sequence of SEQ ID NO:2 except that the sequence of said mutant gene has an insertion of a T nucleotide and a deletion of an A nucleotide at the positions corresponding to 15-position and 333-position respectively, from the first nucleotide of the initiation codon.

12. A mutant barnase gene comprising nucleotide sequence of SEQ ID NO:3.

13. A mutant barnase gene comprising a nucleotide sequence modified from that of the mutant barnase gene as set forth in claim 11 by substitution, deletion, insertion or addition of one to several nucleotides, provided that the inserted T nucleotide and the deleted A nucleotide are not modified.

14. A mutant barnase gene as claimed in claim 13, wherein the nucleotides in the region of from 1- to 27-positions of said mutant barnase gene are the same as those in the region from 1- to 27-positions of the mutant barnase gene of claim 12.

15. A mutant barnase gene comprising one of the nucleotide sequences which encode the amino acid sequence of SEQ ID NO:2 except that the sequence of said mutant gene has an insertion of a T nucleotide at the position corresponding to 15-position from the first nucleotide of the initiation codon.

*B  
and*  
16. A mutant barnase gene comprising the nucleotide sequence of SEQ ID NO:1 except that the sequence of said mutant gene has an insertion of a T nucleotide at the position corresponding to 15-position from the first nucleotide of the initiation codon of the nucleotide sequence of SEQ ID NO:1.

17. A mutant barnase gene comprising a nucleotide sequence modified from that of the mutant barnase gene as set forth in claim 15 by substitution, deletion, insertion or addition of one to several nucleotides, provided that the inserted T nucleotide is not modified.

18. A mutant barnase gene as claimed in claim 17, wherein the nucleotides in the region of from 1- to 27-positions of said mutant barnase gene are the same as those in the region from 1- to 27-positions of the mutant barnase gene of claim 16.

19. A mutant barnase gene comprising a nucleotide sequence having a mutation from one of the nucleotide sequences which encode the amino acid sequence of SEQ ID NO:2, wherein said mutation generates a frameshift from the native barnase gene and yet the mutant barnase gene is capable of coding for a protein having barnase activity on account of frameshift re-coding.

*Revised*  
20. A mutant barnase gene comprising a nucleotide sequence having a mutation from the nucleotide sequence of SEQ ID NO:1, wherein said mutation generates a frameshift from the native barnase gene and yet the mutant barnase gene is capable of coding for a protein having barnase activity on account of frameshift re-coding.

21. A mutant barnase gene comprising a nucleotide sequence modified from that of the mutant barnase gene as set forth in claim 19 by substitution, deletion, insertion or addition of one to several nucleotides, provided that the mutation site described in claim 19 is not modified.

22. The mutant barnase gene as claimed in any one of claims 11 to 21, wherein a protein encoded by said mutant barnase gene is

capable, when expressed anther specifically in a plant, of making said plant substantially male sterile without exerting any substantially disadvantageous effect on the tissues except for the anthers.

23. A DNA comprising a mutant barnase gene as set forth in any one of claims 11 to 21 and a promoter located upstream of said gene for allowing an anther-specific expression of said gene, wherein said expression is capable of making a plant substantially male sterile when the DNA is introduced into the genome of said plant.

24. A method to obtain a mutant barnase gene as claimed in any one of claims 19 to 21, comprising the steps of:

- (1) providing variously mutated barnase genes,
- (2) introducing said mutant barnase genes into E. coli cells and selecting colonies which are smaller than that of control E. coli, which do not have any barnase gene,
- (3) determining the nucleotide sequences of the mutant barnase genes in the selected colonies, and
- (4) selecting the mutant barnase gene comprising a frameshift in the coding region of the native barnase gene.

25. The method as claimed in claim 24, wherein the step (4) comprises selecting the mutant barnase gene having a termination codon in the coding region of native barnase gene as a result of said frameshift in the coding region.

26. A recombinant vector which contains a DNA as claimed in claim 23 and expresses said gene encoded by said DNA in a host plant.

27. A method of making a plant male sterile which comprises transforming said plant with a mutant barnase gene encoded by the DNA as claimed in claim 23, and allowing said mutant barnase gene to be expressed anther-specifically.

28. The method as claimed in claim 27, wherein said plant is transformed with a nucleotide sequence encoding said mutant barnase gene by integrating said nucleotide sequence into the genome of said plant.

29. A transgenic plant wherein a gene encoded by DNA as claimed in claim 23 has been introduced. --